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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/729,830	12/05/2003	Florian Von Der Mulbe	22122-00009-US	8653

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EXAMINER	
DUNSTON, JENNIFER ANN	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/729,830	VON DER MULBE ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 December 2009 and 03 February 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *31-37* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *31-37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *05 December 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *8/11/2010*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 12/22/2009 has been entered.

Receipt is acknowledged of an amendment, filed 12/22/2009, in which claim 31 was amended. The amendment to claim 31 was not proper, because matter to be deleted must be shown by strike-through, except that double brackets placed before and after the deleted characters may be used to show deletion of five or fewer characters. See 37 CFR 1.121(c). However, the amendment has been entered. Receipt is also acknowledged of an amendment filed 2/3/2010 in which claim 32 was amended, and claim 37 was newly added. Claims 31-37 are pending in the instant application.

Election/Restrictions

Applicant elected Group I with traverse in the reply filed on 7/1/2005.

Currently, claims 31-37 are under consideration.

Information Disclosure Statement

Receipt of an information disclosure statement, filed on 8/11/2010, is acknowledged. The signed and initialed PTO 1449 has been mailed with this action.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 31-33 and 36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhou et al (Human Gene Therapy, Vol. 10, pages 2719-2724, November 1999, cited as reference CZ on the IDS filed 1/28/2008; see the entire reference) in view of Kim et al (Gene, Vol. 199, pages 293-301, 1997; see the entire reference) and Adema et al (US Patent No. 6,500,919 B1, cited in a prior action; see the entire reference). This is a new rejection.

Zhou et al teach the development of RNA-based cancer vaccines, because RNA has some advantages over DNA, such as its safety factor and reduced potential to integrate into host chromosomes after transfection, as well as its transient expression that makes it relatively easier

to evaluate and control gene expression *in vivo* (e.g., page 2719, paragraph bridging columns). Zhou et al teach that human melanoma-associated antigen gp100 is a melanocyte differentiation antigen recognized by HLA-A-restricted cytotoxic T lymphocytes (CTLs) as well as Ab in patients with melanoma (e.g., page 2719, right column, full paragraph). Zhou et al teach a pharmaceutical composition comprising mRNA that encodes the gp100 human tumor antigen and a pharmaceutically acceptable carrier, where the pharmaceutical composition is formulated for injection (e.g., page 2720, *Preparation of HVJ-liposomes*; page 2720, *gp100 RNA immunization and B16 cell challenge*). Zhou et al teach that the use of the RNA in the disclosed preclinical studies demonstrates that an RNA tumor antigen vaccine strategy has potential application for human cancer treatment and prevention (e.g., Abstract; page 2723, right column).

Zhou et al do not teach the pharmaceutical composition where the gp100 human tumor antigen mRNA has been stabilized by increasing its Guanine/Cytosine (G/C) content relative to that of a wild type mRNA encoding the polypeptide, and do not teach increasing the G/C content at least 7% relative to the wild type mRNA.

Kim et al teach that control of gene expression at the translational level is mostly governed by the coding gene structure, and one way to increase protein yield from mRNA is to modify the coding sequence of an individual gene without altering the amino acid sequence of the gene product (e.g., paragraph bridging pages 293-294). Kim et al teach that it is known that the choice of synonymous codons in many species is strongly biased and that a correlation exists between high expression and the use of selective codons in a given organism (e.g., page 294, paragraph bridging columns). Efficient expression of a codon-optimized gene can be attributed not only to the abundance of isoacceptor tRNAs and modified nucleotides at the anticodon

wobble position available in a host, but also to the formation of a secondary structure of the transcripts favorable for translation (e.g., page 294, paragraph bridging columns). Kim et al teach the preferred codons of highly expressed human genes in Figure 1. Further, Kim et al teach that the human prevalent codons usually have C or G at their third degenerative position and engineering with human codon usage can result in stable mRNA secondary structures because of stronger GC base pairing (e.g., page 294, paragraph bridging columns). Thus, Kim et al teach that codon optimization to preferred human codons increases G/C content of an mRNA and results in a more stable secondary structure. Kim et al teach the optimization of the human EPO mRNA sequence to preferred human codons, except for some positions that were modified to introduce restriction enzyme sites (e.g., page 297, section 3.1; Figure 2). Kim et al compared the expression of the human-optimized transcript with higher G/C content to a yeast-optimized transcript with lower G/C content and found that the human optimized transcript directed the synthesis of human EPO protein more efficiently than the yeast-optimized transcript (e.g., page 297, paragraph bridging columns; Figure 4). Specifically, in human embryonic kidney 293T cells, the human-optimized transcript provided 37.2 U/ml, whereas the yeast-optimized transcript provided 14.7 U/ml (e.g., page 297, paragraph bridging columns). Kim et al teach that re-engineering the coding sequence to match the codons frequently used in human genes is beneficial to achieve high-level expression, and recent reports clearly support this (e.g., page 299, right column).

Adema et al teach the nucleic acid sequence of SEQ ID NO: 1, which encodes the human gp100 polypeptide (e.g., column 4, lines 48-51; Example 1). Adema et al teach that it is well known in the art that the degeneracy of the genetic code permits substitution of bases in a codon

resulting in another codon still coding for the same amino acid (e.g., column 4, lines 52-56; column 5, lines 1-12). Further, Adema et al teach that it is clear that for expression of a gp100 polypeptide with an amino acid sequence shown in SEQ ID NO: 2 use can be made of a derivate nucleic acid sequence with such an alternative codon composition thereby differing from the nucleotide sequence shown in SEQ ID NO: 1 (e.g., column 4, lines 52-60). Moreover, Adema et al teach that the vaccine can be composed of pure DNA, for example, a vector or virus having the DNA sequence encoding the gp100 antigen (e.g., column 10, lines 24-31). Adema et al teach that this vaccine will stimulate formation of cytotoxic T lymphocytes (e.g., column 10, lines 24-31). The codon frequencies of the coding portion of the nucleic acid sequence of SEQ ID NO: 1 are shown in Exhibit I (mailed 12/23/2008).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the gp100 human tumor antigen mRNA of Zhou et al to include the preferred human codons as taught by Kim et al, where the original codon and preferred codon code for the same amino acid, because Kim et al teach it is within the ordinary skill in the art to optimize a human coding sequence with preferred human codons that code for the same amino acid. Furthermore, Kim et al teach that optimization to human codons increases the G/C content of the message and increases the stability of the message because of stronger GC base pairing.

One would have been motivated to make such a modification in order to receive the expected benefit of increasing protein yield from the mRNA as taught by Kim et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

With regard to claim 32, it would have been obvious to increase the G/C content of the gp100 coding sequence by at least 7% relative to that of a wild type mRNA encoding the gp100 tumor antigen, because replacement of most codons with the preferred codons, as taught by Kim et al, results in at least a 7% increase in G/C content relative to the sequence of SEQ ID NO: 1 taught by Adema et al. This figure was obtained by comparing the codon usage shown in Exhibit I (mailed 12/23/2008) to the preferred codons of Kim et al and calculating the number of changes of A/T to G/C.

Claims 34 and 37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhou et al (Human Gene Therapy, Vol. 10, pages 2719-2724, November 1999, cited as reference CZ on the IDS filed 1/28/2008; see the entire reference) in view of Kim et al (Gene, Vol. 199, pages 293-301, 1997; see the entire reference) and Adema et al (US Patent No. 6,500,919 B1, cited in a prior action; see the entire reference as applied to claims 31-33 and 36 above, and further in view of Seldon et al (WO 02/064799 A2, cited as reference BB on the IDS filed 8/11/2010; see the entire reference). This is a new rejection.

The combined teachings of Zhou et al, Kim et al and Adema et al are described above and applied as before.

Zhou et al, Kim et al and Adema et al do not teach gp100 mRNA where all codons are replaced by preferred codons for abundant tRNAs.

Seldon et al teach that an approach to increasing yield using recombinant DNA technology is to modify the coding sequence of a protein of interest without altering the amino acid sequence of the gene product, specifically by replacing codons that are not so frequently

used with codons which are overrepresented in highly expressed mammalian genes (e.g., paragraph bridging pages 2-3). Seldon et al teach that a gene can be synthesized with 100% optimal codons (e.g., page 4, 4th and 5th paragraph; page 26, 2nd full paragraph; page 46; Table 1). Seldon et al teach that the starting point for optimization of an mRNA may be a coding sequence with 100% common codons or a sequence that contains a mixture of common and non-common codons (e.g., page 27, 1st full paragraph).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to optimize the entire coding sequence to provide maximal G/C content by modifying the third degenerative position to contain G or C, because Kim et al demonstrate that expression from the mRNA can be achieved with many different variations in codon optimization in mammalian cells lines (all human changes, except for restriction sites; all yeast, or a combination of yeast and human; e.g., Figures 4-7). Furthermore, Seldon et al teach that optimization of an mRNA may start by making a sequence with 100% common codons or a sequence with a mixture of common and non-common codons. In this case, one would be choosing from a finite number of identifiable solutions with a reasonable expectation of success in achieving expression of the gp100 protein from the modified mRNA. Optimization of all codons would result in a gp100 message with a G/C content increased at least 15% relative to the wild type sequence. This figure was obtained by comparing the codon usage shown in Exhibit I (mailed 12/23/2008) to the preferred codons of Kim et al and Seldon et al and calculating the number of changes of A/T to G/C.

Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Zhou et al (Human Gene Therapy, Vol. 10, pages 2719-2724, November 1999, cited as reference CZ on the IDS filed 1/28/2008; see the entire reference) in view of Kim et al (Gene, Vol. 199, pages 293-301, 1997; see the entire reference) and Adema et al (US Patent No. 6,500,919 B1, cited in a prior action; see the entire reference as applied to claims 31-33 and 36 above, and further in view of You et al (Cancer Research, Vol. 61, pages 197-205, January 2001; see the entire reference). This is a new rejection.

The combined teachings of Zhou et al, Kim et al and Adema et al are described above and applied as before.

Zhou et al, Kim et al and Adema et al do not teach gp100 mRNA further comprising a secretory leader.

You et al teach linking a tumor antigen gene to a leader sequence at its NH_2 terminus for secretion and a cell-binding domain at its COOH terminus for receptor-mediated internalization (e.g., page 197, right column, full paragraph; Figures 1 and 6). You et al refer to these modifications as a retrogen strategy. You et al teach that the retrogen strategy can efficiently cross-present an intracellular tumor antigen to both MHC class II and class I by dendritic cells (DCs) in a cognate manner, leading to the activation of both antigen-specific Th and CTL responses (e.g., page 203, paragraph bridging columns; Figure 6). You et al teach that this strategy can induce broad and potent antitumor immunity and could be generally used to improve the efficacy of tumor vaccines and immunotherapies (e.g., page 203, paragraph bridging columns).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the combined teachings of Zhou et al, Kim et al and Adema to include the secretory leader and receptor-mediated internalization sequences taught by You et al because You et al teach it is within the ordinary skill in the art to use these sequences in combination with a tumor antigen and Zhou et al, Kim et al and Adema et al teach an mRNA encoding a tumor antigen.

One would have been motivated to make such a modification in order to receive the expected benefit of providing an mRNA capable of inducing a broad and potent antitumor immunity by cross-presenting the gp100 tumor antigen to MHC class II and class I by DCs as taught by You et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 31-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Felgner et al (US Patent No. 5,580,859, cited in a prior action; see the entire reference) in view of Zhou et al (Human Gene Therapy, Vol. 10, pages 2719-2724, November 1999, cited as reference CZ on the IDS filed 1/28/2008; see the entire reference), Adema et al (US Patent No. 6,500,919 B1, cited in a prior action; see the entire reference); Nagata et al (Biochemical and Biophysical Research Communications, Vol. 261, pages 445-451, 1999, cited in a prior action; see the entire reference), and Fomsgaard (WO 00/29561 A2, cited in a prior action; see the entire reference). This rejection was made over claims 31-36 in the Office action mailed 12/23/2008 and has been extended to new claim 37.

Felgner et al teach pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a modified mRNA that encodes a polypeptide, wherein the modified mRNA and wild type mRNA encode a polypeptide having an identical amino acid sequence (e.g. column 4, lines 32-45; column 5, lines 7-20; column 8, lines 28-29). Modifications taught by Felgner et al include capping the mRNA, circularizing the mRNA, or chemically blocking the 5' end of the mRNA (e.g. column 9, lines 14-27). Felgner et al teach pharmaceutical compositions comprising a modified mRNA molecule encoding a tumor antigen (e.g., column 8, lines 28-65; column 21, lines 56-67). Felgner et al teach the composition where the mRNA is designed to encode a secreted tumor antigen, which necessarily comprise a secretory leader (e.g., column 19, lines 13-16; column 20, lines 54-58). Felgner et al teach the composition is formulated for injections (e.g., column 4, lines 20-32; column 8, lines 30-32; column 9, lines 42-50; column 23, lines 27-46). Felgner et al teach that the pharmaceutical products are for administration to a human, for example (e.g., column 5, lines 7-20).

Felgner et al do not specifically teach that the tumor antigens encoded by the modified mRNA are human tumor antigens. Felgner et al do not teach the composition where the mRNA has (i) increased GC content relative to that of a wild type mRNA encoding the polypeptide, (ii) G/C content increased at least 15% relative to that of a wild type mRNA encoding the tumor antigen, (iii) maximum GC content, and (iv) substitution of all rare codons with codons recognized by abundant cellular tRNAs.

Zhou et al teach a pharmaceutical compositions comprising mRNA encoding human gp100 melanoma-associated antigen amplified from the established melanoma cell line M12 (e.g., Title; page 2720, Plasmid Preparation; page 2720, gp100 RNA immunization and B16 cell

challenge). Zhou et al teach that RNA-based cancer vaccines have some advantages over DNA, such as its safety factor and reduced potential to integrate into host chromosomes after transfection (e.g., page 2719, paragraph bridging columns). Zhou et al teach that the human melanoma-associated antigen gp100 is a melanocyte differentiation antigen recognized by HLA-A-restricted CTLs as well as antibodies in patients with melanoma (e.g., page 2719, right column). Further, Zhou et al demonstrate that mRNA encoding gp100 is capable of inducing an antibody and CTL response in mice challenged with B16 melanoma cells (e.g., page 2723). Zhou et al teach that the mRNA cancer vaccination strategy provides temporary and rapid production of protein for several days and provides an alternative approach to immunization, compared with peptides and proteins, in that it allows tumor antigens to be expressed in a normal cell background (e.g., page 2723, right column, paragraph 2).

Adema et al teach the nucleic acid sequence of SEQ ID NO: 1, which encodes the human gp100 polypeptide (e.g., column 4, lines 48-51; Example 1). Adema et al teach that it is well known in the art that the degeneracy of the genetic code permits substitution of bases in a codon resulting in another codon still coding for the same amino acid (e.g., column 4, lines 52-56; column 5, lines 1-12). Further, Adema et al teach that it is clear that for expression of a gp100 polypeptide with an amino acid sequence shown in SEQ ID NO: 2 use can be made of a derivate nucleic acid sequence with such an alternative codon composition thereby differing from the nucleotide sequence shown in SEQ ID NO: 1 (e.g., column 4, lines 52-60). Moreover, Adema et al teach that the vaccine can be composed of pure DNA, for example, a vector or virus having the DNA sequence encoding the gp100 antigen (e.g., column 10, lines 24-31). Adema et al teach that this vaccine will stimulate formation of cytotoxic T lymphocytes (e.g., column 10, lines 24-

31). The codon frequencies of the coding portion of the nucleic acid sequence of SEQ ID NO: 1 are shown in Exhibit I (mailed 12/23/2008).

Nagata et al teach that DNA immunization using the gene codon-optimized to mammals through the entire region is very effective (e.g., Abstract). Nagata et al teach that the translational efficiency of codon-substituted genes in mammalian cells is not proportional to, but does correlate with the codon adaptation index (CAI) values of the genes in mammals, although there are some exceptions, and that subsequently the polypeptide expression level in mammalian cells induces specific CTL induction levels in the mouse (e.g., page 450, right column, paragraph 3). Nagata et al state, "Taken together, our results here suggests that the polypeptide expression level becomes much higher when overall codons inserted into the expression plasmid are substituted to the optimal codons, and that DNA immunization with such a plasmid will result in inducing much better immunological reactions as shown in Fig. 3." See page 450, right column, last full paragraph.

Fomsgaard teaches that it was known in the art that rare codons cause pausing of the ribosome, which leads to a failure in completing the nascent polypeptide chain and an uncoupling of transcription and translation (e.g., page 1, lines 30-32). Pausing of the ribosome is thought to lead to exposure of the 3' end of the mRNA to cellular ribonucleases (e.g., page 1, lines 32-33). Fomsgaard teaches that it has been shown that an exchange of the H IV codon usage to that of highly expressed mammalian genes greatly improves the expression in mammalian cell lines (e.g., page 1, lines 27-29). Fomsgaard teaches the construction of a second nucleotide sequence based on a first nucleotide sequence, where the same amino acid sequence encoded by the first and second nucleotide, and the second sequence is designed using the most

frequent codons from highly expressed proteins in mammals, which are shown in Figure 1.

Figure 1 shows the following:

Amino acid	One letter amino acid code	Three letter amino acid code	Codon
Alanine	A	Ala	GCC
Arginine	R	Arg	CGC
Aspargine	N	Asn	AAC
Aspartic acid	D	Asp	GAC
Cysteine	C	Cys	TGC
Glutamine	Q	Gln	CAG
Glutamic acid	E	Glu	GAG
Glycine	G	Gly	GGC
Histidine	H	His	CAC
Isoleucine	I	Ile	ATC
Leucine	L	Leu	CTG
Lysine	K	Lys	AAG
Proline	P	Pro	CCC
Phenylalanine	F	Phe	TTC
Serine	S	Ser	AGC
Threonine	T	Thr	ACC
Tyrosine	Y	Tyr	TAC
Valine	V	Val	GTG

Comparing the preferred codons to all possible codons disclosed in Figure 7 (shown below), it is clear that the preferred codon for each amino acid has maximal GC content as compared to all possible codons for the same amino acid.

aa		Σ	codons
A	Ala	GCX	GCT GCC GCG GCA
C	Cys	TGY	TGT TGC
D	Asp	GAY	GAT GAC
E	Glu	GAR	GAG GAA
F	Phe	TTY	TTT TTC
G	Gly	GGX	GGT GGC GGG GGA
H	His	CAY	CAT CAC
I	Ile	ATH	ATT ATC ATA
K	Lys	AAR	AAG AAA
L	Leu	YTX	TTG TTA CTT CTC CTG CTA
M	Met	ATG	ATG
N	Asn	AAY	AAT AAC
P	Pro	CCX	CCT CCC CCG CCA
Q	Gln	CAR	CAG CAA
R	Arg	MGX	CGT CGC CGG CGA AGG AGA
S	Ser	WSX	TCT TCC TCG TCA AGT AGC
T	Thr	ACX	ACT ACC ACG ACA
V	Val	GTX	GTT GTC GTG GTA
W	Trp	TGG	TGG
Y	Tyr	TAY	TAT TAC

Thus, when all codons are replaced with preferred codons, the coding sequence has maximal GC content while encoding the same polypeptide sequence as the starting nucleic acid sequence.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the mRNA vaccine compositions encoding a tumor antigen protein of Felgner et al to specifically encode human gp100 melanoma-associated antigen taught by Zhou et al and Adema et al, and to include codon optimization across the entire coding sequence as taught by Nagata et al and Fomsgaard, because Zhou et al teach it is within the skill of the art to make a pharmaceutical composition comprising an mRNA encoding human gp100, Adema et al teach it is within the skill of the art to modify the sequence of SEQ ID NO: 1 to encode the same gp100 protein, and Nagata et al teach it is within the ordinary skill in the art to optimize codons of a nucleic acid encoding an antigenic protein. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to replace all codons with the preferred codons taught by Fomsgaard, because Felgner et al, Nagata et al, and Fomsgaard teach modified nucleic acid sequence. Using the known technique of codon optimization to codons recognized by abundant tRNAs in human cells would have been obvious to one of ordinary skill. Moreover, it would have been obvious to increase the G/C content of the gp100 coding sequence by at least 15% relative to that of a wild type mRNA encoding the gp100 tumor antigen, because replacement of all codons with the preferred codons taught by Fomsgaard results in a 16% increase in G/C content relative to the sequence of SEQ ID NO: 1 taught by Adema et al. This figure was obtained by comparing the codon usage shown in Exhibit I to the preferred codons of Fomsgaard and calculating the number of changes of A/T to G/C. Replacement with all rare codons with the preferred codons of Fomsgaard results in maximal G/C content, because the

preferred codons have the highest C/C content possible while still coding for the same amino acid.

One would have been motivated to make such a modification in order to receive the expected benefit of increasing the expression of the antigenic protein, as taught by Nagata et al and Fomsgaard, and to provide a better immunological response as taught by Nagata et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Response to Amendment -- Declaration of Dr. Hoerr

The declaration under 37 CFR 1.132 filed 12/22/2009 is insufficient to overcome the rejection of claims 31-37 based upon application of the Felgner et al, Zhou et al, Adema et al, Nagata et al, and Fomsgaard references applied under 35 U.S.C. 103(a) as set forth in the last Office action because: the declaration does not provide sufficient evidence of unexpected results or of a lack of rationale to combine the teachings of the references.

Paragraphs 2-7 of the declaration are directed to evidence of increased stability of mRNA with enriched G/C content. The delcarant asserts that this increased stability of the mRNA was unexpected. At paragraph 5, the declaration points to a review by Koide et al, which states the following:

> Although mRNA might be highly attractive owing to the tack of potential risk of integration into the genome, it does not seem very promising as a general method. The main reason seems to be the instability of the mRNA.

The declaration provides this statement as evidence of the unexpectedness of increased stability.

The assertion that the increased stability was unexpected is not found persuasive. It was known in the art at the time the invention was made that increasing the GC content of an mRNA stabilizes the mRNA because of stronger GC base pairing (Kim et al. Gene, Vol. 199, pages 293-301, 1997; e.g., page 294, paragraph bridging columns). Accordingly, this property would not have been unexpected. Furthermore, one of skill in the art would have used the teachings of Koide et al as motivation to solve the problem of instability by increasing the G/C content to provide greater stability and/or expression as taught by the prior art cited in the rejections of record.

At paragraphs 8-10, the declaration states that increased mRNA stability and increased expression based upon codon optimization are distinct concepts. The declaration asserts that codon optimization may eventually but in no way necessarily result in an increased G/C content of DNA (and therefore the mRNA transcribed therefrom). The declaration states that the most preferred codon for arginine in human cells is AGA although codons AGG, CGU, CGC, CGA and CGG, exhibiting higher G/C content may be selected due to the degenerated genetic code. The declaration points to Exhibit C for support of this statement.

This argument is not found persuasive. See pages 6-7 of the Office action mailed 12/23/2008. In every case, the preferred codon used for codon optimization has the maximal G/C content to code for the particular amino acid. For example, the preferred codon for alanine is GCC. All possible codons coding for alanine are GCT, GCC, GCG, GCA. Thus, the preferred GCC codon has the maximal G/C content. This is true for all codons, including arginine. Fomsgaard teaches that the preferred codon for arginine is CGC. This is consistent with the teachings of Kim et al and Seldon et al. Kim et al teach that the CGC codon is used

37% of the time (a percentage that is greater than any other codon that could be used) when looking at highly expressed human genes (e.g., Figure 1). Seldon et al also teach that the CGC codon is used 37% of the time in highly expressed human genes (Table 1). Exhibit C appears to be a codon usage database of all human sequences. One seeking to optimize codon usage would only be interested in the codons used in highly expressed human genes, as taught by the art of record. Thus, the assertion that codon optimization to preferred human codons in no way necessarily result in an increased G/C content is incorrect. One using the preferred codons taught by the prior art (e.g., Fomsgaard et al, Kim et al, and Seldon et al) would necessarily be increasing the G/C content of an mRNA by selecting preferred codons coding for the same amino acid.

At paragraphs 11-15 and 17, the declaration asserts that there is no suggestion in Fomsgaard or Nagata et al to optimize codons in non-HIV-1 genes, let alone human genes. At paragraph 16, the declaration notes that human genes are not dependent on Rev for expression. At paragraph 18, the declaration points to the portion of the Koide et al review (Exhibit D) that states the following:

> Although many bacteria have been targets of DNA vaccines, significant progress has been made with Mycobacterium tuberculosis. One of the reasons seems to be the bias in codon usage in M. tuberculosis genes. Surprisingly, the bias, unlike any other bacteria examined, is comparable to that of Mus musculus and Homo Sapiens (17), suggesting that the codon-optimization described above could be unnecessary for the construction of DNA vaccines against M. tuberculosis.

The declaration asserts that codon optimization was not considered to relevant or necessary in the case of genes already having mammalian codon bias. At paragraph 19, the declaration points

to Exhibits F and G as further evidence that one would only optimize codons for sequences from pathogenic organisms or heterologous genes poorly expressed in mammalian cells.

These arguments are not found persuasive. Once differences are identified between the claimed invention and the prior art, those differences must be assessed and resolved in light of the knowledge possessed by a person of ordinary skill in the art. One of skill in the art would have known that optimization to preferred human codons could be applied to human mRNA sequences. Optimization of human coding sequences to preferred human codons was taught in the prior art (Kim et al. Gene, Vol. 199, pages 293-301, 1997, which teaches optimization of a human EPO cDNA sequence to preferred human codons; Seldon et al. WO 02/064799, cited on the IDS filed 8/11/2010; e.g., optimization of human genes, such as Factor VIII, for expression in human cells, where all codons are optimized, see page 4, 4th and 5th paragraphs, pages 8-10 and 20-22, page 31, 1st full paragraph). Seldon et al state the following with regard to optimization of human codons from a human mRNA sequence for expression in a human expression system (see page 32, last paragraph):

> A large fraction of the codons in the human messages encoding Factor VIII and Factor IX are non-common codons or less common codons. Replacement of at least 98% of these codons with common codons will yield nucleic acid sequences capable of higher level expression in a cell culture. Preferably, all of the codons are replaced with common codons and such replacement results in at least a 2 to 5 fold, more preferably a 10 fold and most preferably a 20 fold increase in expression when compared to an expression of the corresponding native sequence in the same expression system.

Accordingly, the prior art clearly teaches the "optimization" of a human coding sequence with preferred human codons for expression in a human cell. "A person of ordinary skill in the art is also a person of ordinary creativity, not an automaton." KSR, 550 U.S. 398, 127 S. Ct. 1727, 82

*U.S.P.Q.*2d 1385. One of ordinary skill in the art would have seen the benefit of applying a human codon optimization scheme to a human coding sequence. Furthermore, it is noted that the claims do not exclude the optimization of pathogen sequences. The claims require the optimized sequence to encode a "human tumour antigen." The specification discloses that the term "human tumour antigen" encompasses sequences that encode pathogenic viral sequences, such as the human papillomavirus (HPV) E7 protein (e.g., paragraph [0052]).

At paragraph 20, the declaration notes that the cited references of Fomsgaard and Nagata disclose the codon optimization of DNA, not mRNA. However, the declaration does acknowledge that DNA is transcribed to mRNA. The declaration goes on to assert that neither Fomsgaard nor Nagata suggests modification of an mRNA in the isolated environment of a pharmaceutical composition.

These arguments are not found persuasive. By modifying the DNA, the mRNA transcribed from the RNA will necessarily have those same modifications. Furthermore, the modifications were made in the prior art to improve translation which is preformed using the mRNA and not the DNA as a template. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In the instant case, the pharmaceutical composition comprising the isolated mRNA is taught by the Felgner reference.

The declaration has been fully considered but is not deemed persuasive in view of the record as a whole. Therefore, the claims stand rejected under 35 U.S.C. 103(a).

Response to Arguments - 35 USC § 103

With respect to the rejection of claims 31-37 under 35 U.S.C. 103(a) as being unpatentable over Felgner et al in view of Zhou et al, Adema et al, Nagata et al, and Fomsgaard, Applicant's arguments filed 12/22/2009 have been fully considered but they are not persuasive.

The response asserts that the claimed invention would not have been obvious for at least the following reasons: 1) the inventors surprisingly found that an mRNA which is G/C enriched is more stable than the corresponding wild-type mRNA; and 2) and at the time of the invention, it would not have been obvious to enrich the G/C content of an mRNA encoding a human tumor antigen for expression in a human system.

Regarding the first point, the response indicates that Dr. Hoerr's declaration provides further evidence confirming the disclosure of the specification that G/C enrichment unexpectedly stabilizes mRNA.

This argument is not found persuasive. It was known in the art at the time the invention was made that increasing the GC content of an mRNA stabilizes the mRNA because of stronger GC base pairing (Kim et al. Gene, Vol. 199, pages 293-301, 1997; e.g., page 294, paragraph bridging columns). Accordingly, this property would not have been unexpected.

Regarding the second point, Applicants stress the importance of viewing the "state of the art" as it would have been understood by persons actually working in the art at the time the invention was made, avoiding hindsight. The response asserts that one would have seen no reason to "optimize" the codons of a human gene for expression in a human cell, and the cited art

is directed to optimization of poorly-expressed pathogenic, non-human genes in human cells in order to improve vaccine efficacy against pathogens.

This argument is not found persuasive. The prior art teaches the "optimization" of human coding sequences to preferred human codons (Kim et al. Gene, Vol. 199, pages 293-301, 1997, which teaches optimization of a human EPO cDNA sequence to preferred human codons; Seldon et al. WO 02/064799, cited on the IDS filed 8/11/2010; e.g., optimization of human genes, such as Factor VIII, for expression in human cells, where all codons are optimized, see page 4, 4th and 5th paragraphs, pages 8-10 and 20-22, page 31, 1st full paragraph). Seldon et al state the following with regard to optimization of human codons from a human mRNA sequence for expression in a human expression system (see page 32, last paragraph):

> A large fraction of the codons in the human messages encoding Factor VIII and Factor IX are non-common codons or less common codons. Replacement of at least 98% of these codons with common codons will yield nucleic acid sequences capable of higher level expression in a cell culture. Preferably, all of the codons are replaced with common codons and such replacement results in at least a 2 to 5 fold, more preferably a 10 fold and most preferably a 20 fold increase in expression when compared to an expression of the corresponding native sequence in the same expression system.

Accordingly, the prior art clearly teaches the "optimization" of a human coding sequence with preferred human codons for expression in a human cell. In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include

knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

The response notes that Adema et al do not suggest the use of G/C-enriched molecules and does not suggest any benefits associated with using G/C-enriched molecules. Further, the response asserts that Adema et al do not supply any motivation to modify the disclosure of Felgner et al and/or Zhou et al to enrich the G/C content of an mRNA encoding a human tumor antigen.

This argument is not found persuasive. The rejection of record is based upon the combined teachings of Felgner et al, Zhou et al, Adema et al, Nagata et al, and Fomsgaard. Fomsgaard et al teach that it was known in the art that rare codons cause pausing of the ribosome, which leads to a failure in completing the nascent polypeptide chain (e.g., page 1, lines 30-32). Further, Fomsgaard et al teach the most frequent codons from highly expressed proteins in mammals (Figure 1). One would have been motivated to make such a modification to increase expression of the encoded protein, or specifically the encoded tumor antigen.

The response notes that Nagata co-authored a review article published in 2000, which Applicant asserts is suggestive of the non-obviousness of the present invention. The response points to the portion of the review that states the following:

> Although many bacteria have been targets of DNA vaccines, significant progress has been made with Mycobacterium tuberculosis. One of the reasons seems to be the bias in codon usage in M. tuberculosis genes. Surprisingly, the bias, unlike any other bacteria examined, is comparable to that of Mus musculus and Homo Sapiens (17), suggesting that the codon-optimization described above could be unnecessary for the construction of DNA vaccines against M. tuberculosis.

The response asserts that "codon optimization was not considered to relevant or necessary in the case of genes already having mammalian codon bias. Further, the response points to Fomsgaard's disclosure as being concerned with optimizing HIV and rendering genes rev-independent, and states that "codon re-engineering" is applicable only in the case of "proteins that are poorly expressed in mammalian cells." Moreover, the response asserts that it would be counterintuitive to optimize human genes for expression in human cells, because it goes against millions of years of evolution. The response also asserts that no correlation between codon usage and expression level in human genes was understood at the time of the present invention.

This argument is not found persuasive in vie of the teachings of Kim et al and Seldon et al, which are discussed above. These references demonstrate that codon optimization is not necessary but can be used to stabilize and increase the expression of a protein from an mRNA.

The response asserts that codon optimization does not necessarily increase G/C content.

This argument is not found persuasive. See pages 6-7 of the Office action mailed 12/23/2008. In every case, the preferred codon used for codon optimization has the maximal G/C content to code for the particular amino acid. For example, the preferred codon for alanine is GCC. All possible codons coding for alanine are GCT, GCC, GCG, GCA. Thus, the preferred GCC codon has the maximal G/C content. This is true for all codons.

The response asserts that codon-optimization to minimize ribosomal pausing would not have been recognized as relevant to expression of human genes in human cells.

This argument is not found persuasive. Fomsgaard teaches the construction of a nucleotide sequence, where the sequence is designed using the most frequent codons from highly expressed proteins in humans, which are shown in Figure 1. Even if ribosomal pausing is not

relevant, one would have recognized that certain codons are preferred to obtain higher expression of a protein in a human cell.

The response asserts that Fomsgaard and Nagata et al disclose the codon optimization of DNA not RNA and do not suggest to provide a modified mRNA.

This argument is not found persuasive. By modifying the DNA, the mRNA transcribed from the RNA will necessarily have those same modifications. Furthermore, the modifications were made in the prior art to improve translation which is preformed using the mRNA and not the DNA as a template.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is (571)272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached on 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Jennifer Dunston/
Primary Examiner
Art Unit 1636